Appendix I – Clean Copy of Pending Claims

1. (Amended) A modified serine hydrolase, said hydrolase comprising an amino acid residue in a subsite replaced with a cysteine, wherein the cysteine is modified by replacing the thiol hydrogen in the cysteine with a substituent group providing a thiol side chain comprising a chiral substituent.

2. The modified serine hydrolase of claim 1, wherein the serine hydrolase catalyzes a transamidation.

3. The modified serine hydrolase of claim 1, wherein the serine hydrolase catalyzes a transpeptidation.

4. The modified serine hydrolase of claim 1, wherein the serine hydrolase catalyzes a transesterification.

5. The modified serine hydrolase of claim 1, wherein said serine hydrolase is selected from the group consisting of an alpha/beta serine hydrolase, a subtilisin type serine protease, and a chymotrypsin serine protease.

6. The modified serine hydrolase of claim 1, wherein said serine hydrolase is a subtilisin.

7. The modified serine hydrolase of claim 6, wherein said serine hydrolase catalyzes a transamidation and is stereoselective.

8. The modified serine hydrolase of claim 6, wherein the amino acid replaced with a cysteine is an amino acid in the S_1, S_1', or S_2 subsite.

9. The modified serine hydrolase of claim 8, wherein the amino acid replaced with a cysteine is selected from the group consisting of asparagine, leucine, methionine, and serine.

10. (Amended) The modified serine hydrolase of claim 8, wherein said amino acid is selected from the group consisting of amino acid 156 in the S_1 subsite, amino acid 166 in the S_1 subsite, amino acid 217 in the S_1' subsite, amino acid 222 in S_1' subsite and amino acid 62 in the S_2 subsite.

11. (Amended) The modified serine hydrolase of claim 8, wherein said substituent is selected from the group consisting of an enantiomerically pure oxazolidinone, an enantiomerically pure indenone, and an enantiomerically pure phenyl-ethyl-thiol.

13. (Amended) The modified serine hydrolase of claim 11, wherein said substituent is selected from the group consisting of (R)-2-methoxy-2-phenyl-ethyl-thiol, (S)-2-methoxy-2-phenyl-ethyl-thiol, (R)-2-hydroxy-2-phenyl-ethyl-thiol, (S)-2-hydroxy-2-phenyl-ethyl-thiol, N-(3'-thio-propyl)-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-isopropyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-isopropyl-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-

phenyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-phenyl-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-benzyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(R)-4-phenyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-phenyl-2-oxazolidinone, N-(2'-thioethyl)-(R)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-benzyl-2-oxazolidinone, N-(3'-thio)-(3aR-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one, and N-(3'-thio)-(3aS-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one.

51. (Amended) A method of producing a chemically modified mutated serine hydrolase, said method comprising

providing a serine hydrolase, said hydrolase comprising an amino acid residue in a subsite replaced with a cysteine; and

modifying the cysteine by replacing the thiol hydrogen in the cysteine with a substituent group providing a thiol side chain comprising a chiral substituent.

52. The method of claim 51, wherein said hydrolase is selected from the group consisting of an alpha/beta serine protease, a subtilisin type serine protease, and a chymotrypsin serine protease.

53. The method of claim 51, wherein said hydrolase is a subtilisin.

54. The method of claim 53, wherein the amino acid replaced with a cysteine is an amino acid in the S_1, S_1', or S_2 subsite.

55. The method of claim 53, wherein the amino acid replaced with a cysteine is selected from the group consisting of asparagine, leucine, methionine, and serine.

56. (Amended) The method of claim 53, wherein said amino acid is selected from the group consisting of amino acid 156 in the S_1 subsite, amino acid 166 in the S_1 subsite. amino acid 217 in the S_1' subsite, amino acid 222 in S_1' subsite and amino acid 62 in the S_2 subsite.

57. (Amended) The method of claim 53, wherein said substituent is selected from the group consisting of a chiral oxazolidinone, a chiral indenone, and a chiral phenyl-ethyl-thiol.

59. (Amended) The method of claim 53, wherein said substituent is selected from the group consisting of (R)-2-methoxy-2-phenyl-ethyl-thiol, (S)-2-methoxy-2-phenyl-ethyl-thiol, (R)-2-hydroxy-2-phenyl-ethyl-thiol, (S)-2-hydroxy-2-phenyl-ethyl-thiol, N-(3'-thio-propyl)-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-isopropyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-isopropyl-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-phenyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-phenyl-2-oxazolidinone, N-(3'-thio-propyl)-(R)-4-benzyl-2-oxazolidinone, N-(3'-thio-propyl)-(S)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(R)-4-phenyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-phenyl-2-oxazolidinone, N-(2'-thioethyl)-(R)-4-benzyl-2-oxazolidinone, N-(2'-thio-ethyl)-(S)-4-benzyl-2-oxazolidinone, N-(3'-thio)-(3aR-cis)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]-oxazol-2-one, and N-(3'-thio)-(3aS-cis)-3,3a,8,8a-tetrahydro-2H-

indeno[1,2-d]-oxazol-2-one.

61. The method of claim 53, wherein said method further comprises screening the modified serine hydrolase for an activity selected from the group consisting of a transesterification activity, a transamidation activity, and a transpeptidation activity.

62. The method of claim 61, wherein said activity is stereoselective.